PURIFICATION OF A HUMAN POST-HEPARIN PLASMA TRIGLYCERIDE LIPASE

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1. Introduction

Intravenous injection of heparin releases lipolytic activities against a variety of substrates including triglycerides, diglycerides, monoglycerides and phospholipids [1–5]. Triglyceride lipase (TGL) in post-heparin plasma has been considered to be identical with lipoprotein lipase (LPL), an enzyme present in adipose tissue, heart, mammary gland and other organs [6]. LPL is defined as a triglyceride lipase requiring lipoproteins or apolipoproteins for full activity. When obtained from these tissues, LPL has been shown consistently to be inhibited by 1 M sodium chloride. Unpurified TGL from plasma, however, has been shown by various investigators to be only partially inhibited or even activated by sodium chloride [7–9]. The variable inhibition by high ionic strengths has been attributed to the presence of more than one TGL in plasma [10]. This concept has not been established by purification of multiple enzymes and the possibility remains that the different activities observed may be in part due to differences in assay conditions or other factors. Recently a LPL has been isolated from human post-heparin plasma [11, 12]; however, detailed studies on the effects of NaCl on these preparations are yet to be presented. In this publication we report on studies which led to a highly purified TGL from human post-heparin plasma. This enzyme differed from LPL in rat adipose tissue by failing to show suppressed activity in 1 M sodium chloride and thus is similar to that of a TGL released by heparin from liver.

2. Materials and methods

2.1. *Enzyme assays*

Triglyceride lipase assays were carried out as follows: In a total volume of 0.5 ml each vial contained i) 3.3 nmoles of [1-^{14}C]trioleate (7.3×10^4 dpm); ii) 0.67 μmoles of unlabeled trioleate; iii) 2.5 mg gum arabic; iv) 5 mg bovine albumin; v) 20 μl of post-heparin plasma or variable amounts of enzyme solution. Final buffer concentrations were 0.2 M Tris-HCl (pH 8.4) with concentrations of sodium chloride from 0.01 to 2 M. Incubations were done in duplicate for 30 min at 27°. Total lipids were extracted by the method of Dole and Meinertz and ^{14}C-labeled free fatty acids were isolated on an ion exchange resin by a modification [13] of the method of Kelley [14]. Enzyme activity was calculated as nmoles [^{14}C]FFA/ml/hr. With this system enzyme activity was directly proportional to the amount of up to 40 μl of post-heparin plasma added. [^{14}C]FFA release was linear with time for 1 hr or longer or until 10–12% of the triglyceride was hydrolyzed. Post-heparin plasma monoglyceride hydrolase (MGH) was determined by the method of Greten et al. [2].

2.2. *Enzyme preparation*

Human post-heparin plasma was collected from normal subjects 10 min after intravenous administration of sodium heparin (10 units per kg body weight) [8]. Plasma was adjusted to d = 1.21 g/ml by the addition of KBr and centrifuged for 1 hr at 60,000 rpm in a

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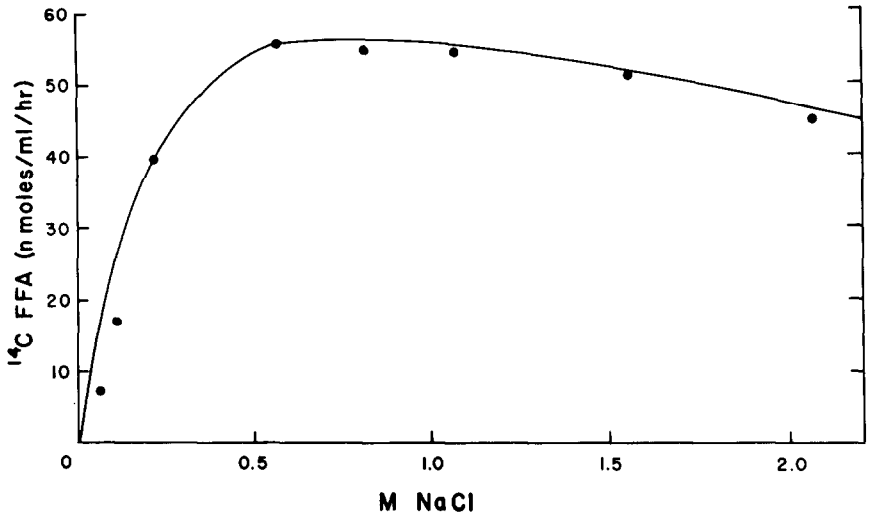


Fig. 1. Effect of NaCl on post-heparin plasma TGL. Representative curve demonstrating the effect of final NaCl concentrations in the incubate on enzyme activity is shown.

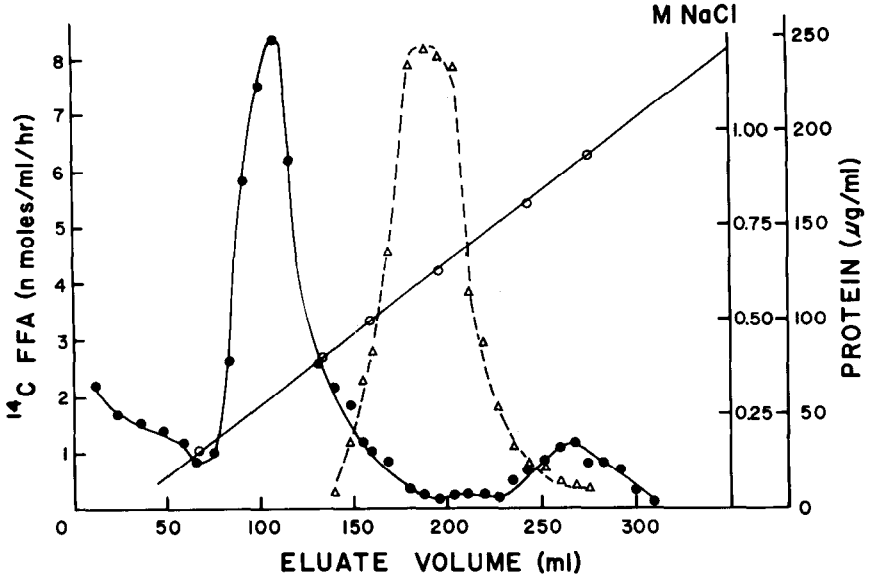


Fig. 2. Affinity chromatography of human post-heparin plasma TGL on heparin-Sepharose. A plasma sample (42 ml) of the d = 1.21 infranatant which had been dialyzed and diluted before (Methods) was applied to the column. 200 ml of the starting buffer were then applied, eluting a large protein peak not shown on the figure. A gradient between 0.15 and 1.5 M NaCl with 0.005 M sodium barbital was then started using a total volume of 400 ml. 4 ml fractions were collected. (•—•—•) Protein measured by the Lowry method. (△—△—△) TGL activity.

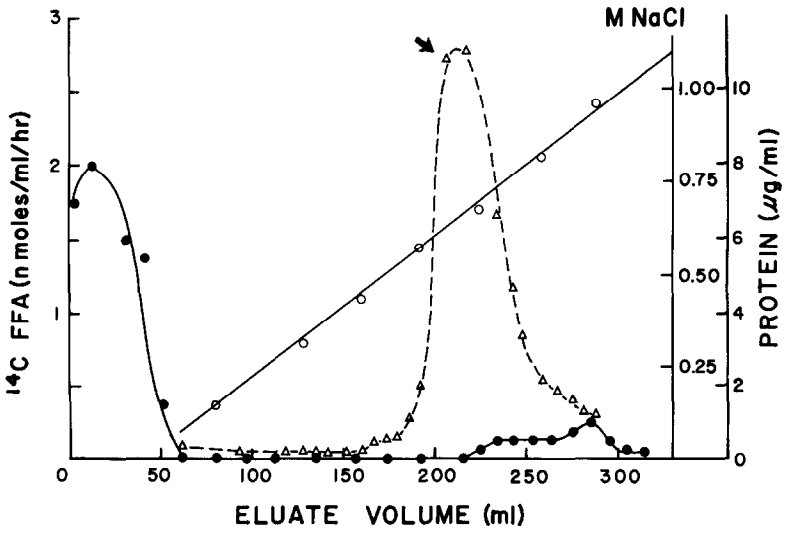


Fig. 3. Affinity chromatography of partially purified TGL on a second heparin-Sepharose column. Fractions between eluate volume 160 and 225 ml from the previous column (fig. 2) were pooled and diluted to the starting buffer concentration. Elution was performed as described in fig. 2. Fraction marked by arrow was used for analysis by polyacrylamide gel electrophoresis (fig. 4).

Spinco model L-2-65B ultracentrifuge using a type 65 rotor. The upper 2 ml were discarded and the infranatant was dialyzed against 0.15 M sodium chloride containing 0.005 M sodium barbital at pH 7.4 for 60 min. The dialyzed plasma preparation was then diluted with an equal volume of 0.15 M sodium chloride in 0.005 M sodium barbital. Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was activated by treatment with cyanogen bromide [15] and heparin was covalently bound to the gel as described by Iverius [16]. The sample was applied to a column of heparin-Sepharose (2.5 × 30 cm) and elution was carried out with a linear gradient of sodium chloride as described in the legend to fig. 2 [17]. Rechromatography of the partially purified enzyme was performed in the same way on a second heparin–Sepharose column after diluting the sample to the conductivity of 0.15 M sodium chloride (conductivity 10 mmho) with 0.005 M sodium barbital.

2.3. *Immunological methods*

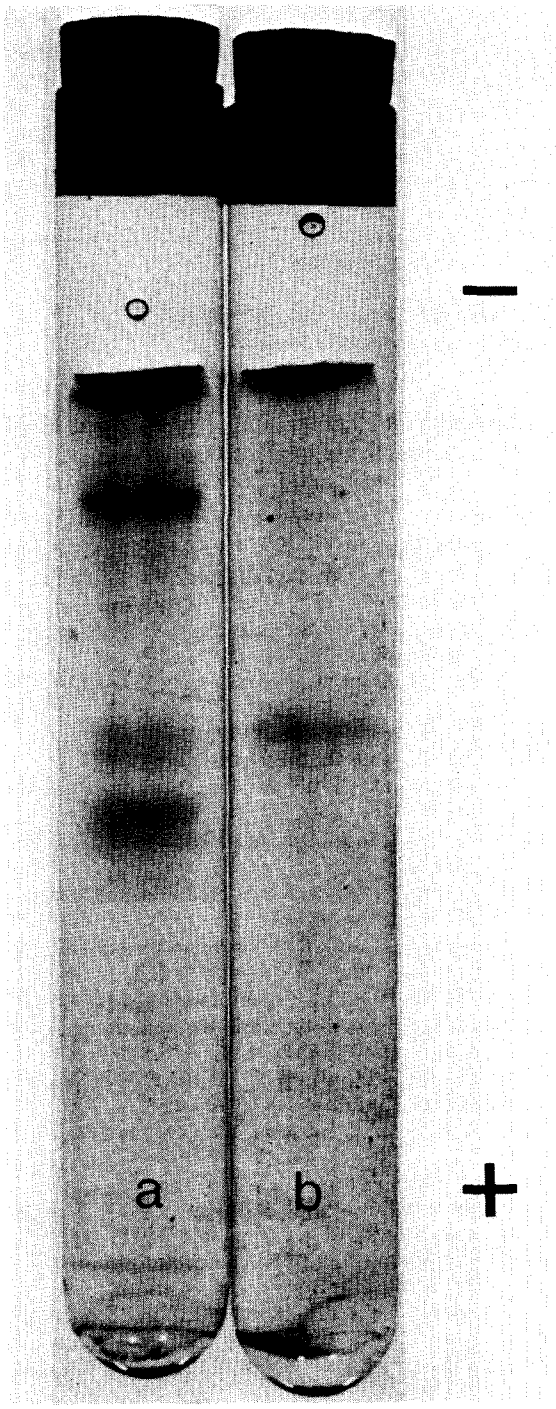
Immunoelectrophoresis was carried out in 1% agarose gel employing barbital buffer, pH 8.6, ionic strength 0.05. Rabbit anti-human serum was prepared in this laboratory for immunochemical identification of protein bands. Double immunodiffusion was carried out by the method of Ouchterlony in the same gel [18].

Polyacrylamide gel electrophoresis was performed in 7.5% acrylamide. The gels were prepared with and without urea (8 M at pH 8.9) using Tris-HCl buffer and protein bands were stained with Coumassie blue [19].

Protein was measured by the method of Lowry et al. with bovine albumin as standard [20].

3. Results

The effects of increasing NaCl concentrations on enzyme activity are shown in fig. 1. Both post-heparin plasma and purified TGL were maximally active between 0.5 and 0.75 M sodium chloride but were only slightly less active at 1 M NaCl. Unless otherwise stated, enzyme assays were carried out at the 1 M sodium chloride concentration. We thus selected conditions which would exclude appreciable measurement of a LPL in plasma which was inactivated at high ionic strengths. Removal of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) from the remaining plasma proteins by ultracentrifugation was found necessary as these lipoproteins also form complexes with heparin. Most of the plasma proteins did



not bind to the column and were eluted during the application of the sample. A much smaller protein peak was observed soon after the initiation of the sodium chloride gradient (fig. 2). The enzyme was then eluted as a single peak of activity with no corresponding protein peak. A third peak of protein appeared after the enzyme activity. Polyacrylamide gel electrophoresis of the enzyme peak showed at least four bands (fig. 4) and by immunoelectrophoresis several precipitin lines were detectable against anti-human plasma. A much greater purification was obtained by rechromatography of those fractions showing the highest specific activity on a second heparin-Sepharose column. This chromatogram is shown in fig. 3. MGH activity eluted with that of TGL. One fraction of this enzyme activity peak indicated in fig. 3 stained as a single band on polyacrylamide gel electrophoresis in 8 M urea (fig. 4). This purified TGL was maximally active between 0.5 and 0.75 M sodium chloride as was post-heparin plasma. Furthermore, addition of pre-heparin plasma was not found necessary for enzymatic activity. The purified enzyme did not react with rabbit anti-human sera on immunoelectrophoresis or double immuno diffusion. The final specific activity was 3750 nmoles [^{14}C]FFA/hr/mg protein, representing approx. 10,000-fold purification from crude post-heparin plasma (table 1).

4. Discussion

A triglyceride lipase has been highly purified from human post-heparin plasma. This enzyme showed optimal activity in high sodium chloride concentrations and was not activated by addition of plasma. These characteristics seem to distinguish this TGL from the lipoprotein lipase purified from rat plasma [21] and bovine milk [17], and from that in adipose tissue or heart. Liver perfusates containing heparin [10, 22] and liver homogenates have been shown to contain a TGL with the properties of the enzyme

Fig. 4. Polyacrylamide gel electrophoresis (7.5%, 8 M urea) of concentrated fractions with TGL activity which were obtained from the first heparin-Sepharose (a) and the second heparin-Sepharose (b) column. A single band is seen in the separation gel (b). Protein staining at the interface between the upper and lower gel probably represents some aggregation of the enzyme, perhaps occurring during the stacking process, since assay of gel slices (without urea) revealed enzyme activity at both sites.

Table 1
Summary of the purification steps for human post-heparin plasma TGL.

	Volume (ml)	Protein (mg)	Total activity (nmoles [^{14}C]FFA/hr)	Specific activity (nmoles [^{14}C]FFA/hr/mg protein)	Purification	Yield (%)
Post-heparin plasma	25	3012	1160	0.39	---	100
d = 1.21 Infranatant*	21	1386	825	0.60	1.5	71
Eluate 1**	60	0.74	352	475	1220	41
Eluate 2***	57	0.03	120	3750	9160	10

Data represent enzyme activity in 1 M sodium chloride. Mean out of three experiments are shown.
* Sample obtained after ultracentrifugation at density 1.21 and after dialysis.
** Fractions collected between eluate volume 160–220 ml from the first heparin-Sepharose column were pooled.
*** Fractions collected between 189–246 ml from the second heparin-Sepharose column were pooled.

purified in this study [10]. It is likely, therefore, that this TGL originates from liver. This enzyme may coexist in post-heparin plasma with other TGL activity which is inhibited by high ionic strengths as suggested by LaRosa [10].

Evidence has been previously presented that post-heparin plasma contains different lipolytic activities against triglycerides and micellar monoglycerides [1, 2]. The purified plasma obtained in this study shows activity against both triglyceride emulsions and micellar monoglyceride solutions. TGL and MGH may represent two interconvertible forms of the enzyme and studies with purified enzyme preparations will be necessary for further clarification.

Clearly, assay conditions play an important role in characterizing the properties of an enzyme. Investigators who have observed sodium chloride inhibition with post-heparin plasma as enzyme source have used different triglyceride emulsions and higher incubation temperatures than those in this study [7, 8, 23]. Conditions required for salt inhibition are presently being investigated in our laboratory. Although pre-heparin plasma did not increase activity of this purified plasma enzyme, studies using isolated lipoproteins and their lipid and apoprotein components will be necessary to determine the need of possible cofactors. Interrelationships between purified plasma TGL and plasma lipoproteins are presently under investigation in our laboratory.

Acknowledgements

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